FINAL REPORT
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Biogeochemical Cycling of Manganese at Hydrothermal Vents

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The interactions between bacteria and metals in the ocean are profoundly important both in environments with high levels of metals, such as hydrothermal vents, as well as in metal-poor oligotrophic environments. These represent the environmental extremes for metal concentrations. Metals affect primary productivity by both metal limitation of phytoplankton growth and by serving as a potential energy source for bacterial (autotrophic) growth. In addition, the highly charged surfaces of metal oxide particles, which, to a large extent, are microbially produced, govern much trace metal and organic geochemistry. The role these oxides surfaces play is evident both in hydrothermal vent plumes (which, because they are dispersed hundreds of kilometers away from venting sources, have a profound influence on the chemistry and ecology of the deep sea) as well as in the photic zone, where trace metals and organics are transported via metal oxides out of surface layer and through the water column (so called "redox-mediated transport").

The focus of our research on this contract was on the biogeochemical cycling of manganese at hydrothermal vents and the contribution of bacteria to this process. This work involved both field and laboratory studies. In brief, our research has shown that the high levels of manganese(II) (Mn(II)) oxidation found in hydrothermal vent environments are mediated by bacteria to a significant degree. We have found that a significant proportion of Mn(II) oxidizing bacterial isolates (predominantly from an anoxic basin) contain the gene for the large subunit of Ribulose-1,5-bisphosphate Carboxylase Oxygenase (RubisCO) suggestive of autotrophy. We hypothesize that Mn(II) oxidizers at the vents are autotrophs and contribute to primary production at hydrothermal vents. We are continuing the RubisCO gene probing on the bacterial isolates obtained from the hydrothermal vent environments as part of an ongoing ONR contract. In addition, we have established an indirect mechanism of Mn(II) oxidation for a second class of hydrothermal vent bacteria.

Field Studies

The goals of these studies were:

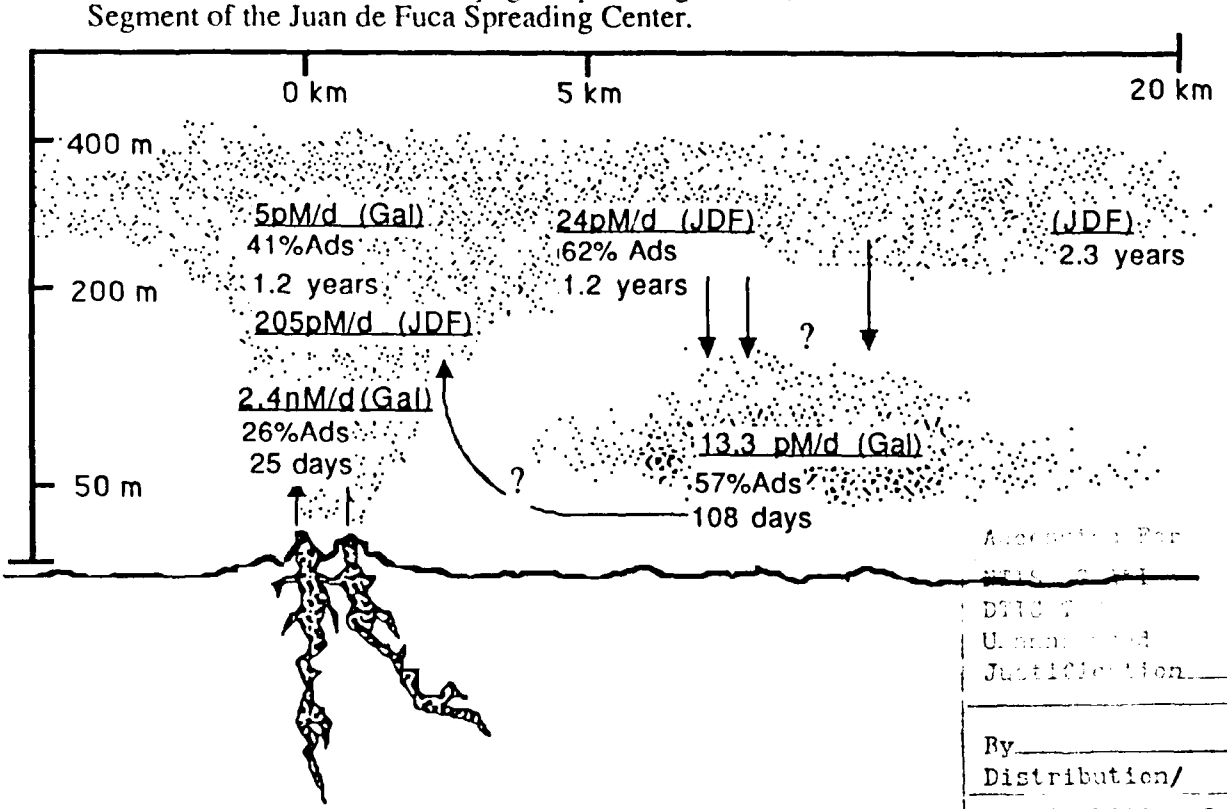
1) to assess the extent of the bacterial contribution to Mn cycling at hydrothermal vents
2) to compare Mn(II) oxidation and adsorption and
3) to measure rates of abiological and microbial Mn(II) oxidation.

Experiments were conducted at two hydrothermal vent locations, the Galapagos and the Juan de Fuca Spreading Centers, to measure the rates of Mn(II) oxidation both near venting water and in the buoyant plumes associated with the vents. Samples at the vents were collected by *ALVIN* and those in the plume were collected by Go-flo bottles mounted on a CTD rosette. In addition, in situ measurements were made within the vent fields at Galapagos and in the plumes both at Galapagos and at the Juan de Fuca sites. Rate measurements were made by using radioactive ^{54}Mn as a tracer to measure the uptake of the dissolved manganese into the particulate phase in the presence and

absence of azide, a poison shown to effectively inhibit microbial manganese oxidation without interfering in the solution chemistry of manganese (Rosson et al., 1984). Samples were incubated with the radiotracer at 2°C (bottom water temperature) and subsamples were removed and filtered through 0.2 μm membrane filters and the total radioactivity and the radioactivity trapped on the filter was counted. In addition, at the end of the incubation, samples were treated with either excess cold dissolved Mn or neutralized ascorbic acid (pH 7.5) to determine what portion of the radioactive particulate manganese was either adsorbed or adsorbed and reducible, respectively (Sunda and Huntsman, 1987). Comparison of the poisoned and unpoisoned samples indicated that microbially mediated manganese oxidation is important within vent fields and within vent plumes, even at distances as great as 20 km from the hydrothermal source. The highest rates of manganese oxidation and fastest turnover times of the dissolved manganese were seen in the vent fields, where the maximal rates were approximately three orders of magnitude greater than those measured in the plumes (Figure.1). In addition, the percentage of the particulate Mn formed in the experiments that was due to adsorbed Mn increased with distance from the hydrothermal source. Thus, in the plumes roughly 40-60% of the particulate Mn is adsorbed Mn(II). In sum, these results suggest that hydrothermal vents are a source of manganese oxidizing bacteria, which may then be dispersed throughout the vent plume, where they may further oxidize Mn(II), albeit at a slower rate.

Figure 1

Measured rates of $^{54}Mn(II)$ binding (underlined; pM/d or nM/d), percent of Mn(II) bound that is adsorbed and not oxidized (exchangeable Mn; Ads) and calculated residence times (days or years) of dissolved Mn with respect to precipitation in regions near hydrothermal vents and with distance in vent plumes. Gal refers to values determined at the Galapagos Spreading Center, JDF refers to the Endeavor Segment of the Juan de Fuca Spreading Center.



Laboratory Studies

The goals of these studies were 1) to isolate the organisms responsible for Mn(II) oxidation at hydrothermal vents and 2) to test the feasibility of using gene probes for Ribulose-1,5-bisphosphate Carboxylase Oxygenase (RubisCO) for identifying autotrophic Mn(II) oxidizing bacteria. The oxidation of manganese(II) has often been considered to be a potential source of energy for the growth of autotrophic or mixotrophic bacteria. Hydrothermal vents, because they provide a large flux of dissolved Mn into oxygenated water, are an ideal environment for these organisms.

Major efforts to isolate Mn(II) oxidizing bacteria from vent samples occurred during two cruises to the Juan de Fuca during Oct 1987 and Aug/Sep 1988. Approximately 45 isolates from the first cruise were obtained in pure culture. Eleven isolates from the second cruise have been purified and more isolates are still being purified. To aid in selecting a potential manganese autotroph for more rigorous physiological characterization, DNA probes for RubisCO were used to screen 45 strains of manganese oxidizing bacteria isolated on Mn(II)-containing inorganic media from a variety of marine environments, including hydrothermal vents and anoxic fjords. The RubisCO probes were developed specifically for detection of autotrophy in manganese oxidizing bacteria during a one year contract (#NOOO14-87-K-0206) from the Molecular Biology Program at ONR. The probes were restriction fragments internal to large subunit genes from *Anacystis nidulans*, *Anabaena* 7120, *Rhodospirillum rubrum* and *Xanthobacter* sp. strain H414, a group of autotrophic organisms that represent a range of RubisCO types. Hybridization conditions were adjusted to the highest stringency that would still allow detection of a *Thiobacillus neapolitanus* positive control with the *Anabaena* probe. Initial colony blots revealed that approximately 20% of the isolates showed some degree of homology with the *A. nidulans* or the *R. rubrum* probes. Some strains showed strong homology with one probe and weak or no homology with the other, while the opposite was true for other strains. This suggests that structurally different RubisCO genes occur in different manganese oxidizers. Based on this initial screening, DNA from several strains was purified and subjected to Southern Blot analysis which confirmed the presence of the RubisCO large subunit gene in these strains. This approach has yielded some strong candidates for physiological assessment of Mn(II) oxidation as an energy source for CO_2 fixation. This work is ongoing as part of an ONR contract.

From the 1987 Juan de Fuca cruise we obtained an unusually avid Mn oxidizing bacterium, strain JDF-1, from direct isolation on a Mn-thiosulfate inorganic medium. JDF87-1 catalyzes manganese oxidation only in the presence of thiosulfate. We have determined that the isolate converts thiosulfate completely to tetrathionate and in the process causes the pH of the medium to rise. Manganese oxidation appears to be catalyzed by this pH increase created during sulfur metabolism. We have compared this isolate with other, better characterized heterotrophic thiosulfate-oxidizing bacteria and found that they also oxidize manganese via tetrathionate production. Since many environments containing reduced sulfur also contain reduced manganese , and heterotrophic thiosulfate-oxidizing bacteria have been shown to be abundant in these environments (particularly at hydrothermal vents), this mechanism may be responsible for some of the catalysis of manganese oxidation *in situ*.

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